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ORIGINAL ARTICLE

In vitro susceptibility of the Gram-negative bacterium *Helicobacter pylori* to extracts of Iranian medicinal plants

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Abstract

The susceptibility of *Helicobacter pylori* to methanol extracts of 12 Iranian medicinal plants used in folk medicine for the treatment of gastric ailments including peptic ulcers disease was screened against one metronidazole-sensitive and one-metronidazole resistant strain of *H. pylori* using the disk diffusion method. Active extracts (zone of inhibition ≥15 mm) were then re-assayed to obtain the minimum inhibitory concentration (MIC) against 12 clinical isolates of *H. pylori* by using the agar dilution method. Extracts of the aerial part of *Artemisia dracunculus* L. (Compositae) and *Teucrium polium* L. (Lamiaceae), leaves of *Salvia mirzayanii* Rech. & Esfand. (Lamiaceae) and *Salvia officinalis* L. (Lamiaceae), flowers of *Zataria multiflora* Boiss. (Lamiaceae), fruits of *Bunium persicum* (Boiss.) B. Fedtsch. (Apiaceae), *Carum carvi* L. (Apiaceae), *Heracleum persicum* Desf. ex Fischer (Apiaceae), *Pimpinella anisum* L. (Apiaceae), *Trachyspermum copticum* (L.) Link (Apiaceae) and *Myrtus communis* L. (Myrtaceae), and seeds of *Nigella sativa* L. (Ranunculaceae) were evaluated in the study. Among them, *S. mirzayanii* had the strongest activity against *H. pylori*, with a MIC of 32 µg/mL.

Keywords: *Anti*-Helicobacter pylori *activity; Iranian medicinal plants; methanol extract*

Introduction

Helicobacter pylori is a human pathogen, infection with which is directly associated with many diseases of the upper gastrointestinal tract, including acute and chronic gastritis, non-ulcer dyspepsia, peptic ulcer disease, and gastric cancers (Williamson, 2001). Treatment of *H. pylori* infection is relatively successful, with usually >80% of patients exhibiting eradication of the organism when combination antibiotic therapies are used (Bytzer & O'Morain, 2005). However, bacterial resistance of *H. pylori* to antibiotics such as clarithromycin and metronidazole has been observed in different parts of the world and continues to increase (Graham & Qureshi, 2000). As a result, there is a need to seek new, safe, and effective anti-*H. pylori* drugs with highly selective antibacterial activity against the pathogen, but without the risk of resistance and untoward effects.

For thousands of years, plant-based medicines have been used to treat gastrointestinal ailments and, thus, plants would seem to be a logical source of new anti-*H. pylori* compounds. In fact, strong *in vitro* evidence suggests that medicinal plants can act as antimicrobial agents against a wide range of bacteria (Mansouri et al., 2001; Hoffman et al., 2004).

In Iran and the Mediterranean area, herbal extracts have been used as traditional medicines and some medicinal plants are used to treat diseases of the upper

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gastrointestinal tract (Foroumadi et al., 2002). The *in vitro* anti-*H. pylori* activities of some Iranian medicinal plants have been previously reported (Malekzadeh et al., 2001; Nariman et al., 2004). Furthermore, the anti-*H. pylori* activities of Greek (Stamatis et al., 2003) and Chinese herbal medicines (Li et al., 2005) have also been reported, as well as that of extracts from Taiwanese folk medicinal plants (Wang & Huang, 2005).

Accordingly, as a part of a screening program for a number of medicinal plants, we have evaluated a series of native Iranian plants, used traditionally for the treatment of peptic ulcer, on clinical isolates of *H. pylori* from adult patients.

Materials and methods

Plant material and extraction procedure

Aerial parts of *Teucrium polium* L. (Lamiaceae) and *Artemisia dracunculus* L. (Compositae), leaves of *Salvia mirzayanii* Rech. & Esfand. (Lamiaceae) and *Salvia officinalis* L. (Lamiaceae), seeds of *Nigella sativa* L. (Ranunculaceae), and flowering tops of *Zataria multiflora* Boiss. (Lamiaceae) were collected from Khabr (Kerman Province, Iran) in June 2002; fruits of *Bunium persicum* (Boiss.) B. Fetsch. (Apiaceae) were collected from the Hezar Mountains (Kerman Province, Iran) in July 2002; and fruits of *Carum carvi* L. (Apiaceae), *Heracleum persicum* Desf. ex Fischer (Apiaceae), *Myrtus communis* L. (Myrtaceae), *Pimpinella anisum* L. (Apiaceae) and *Trachyspermum copticum* (L.) Link (Apiaceae) were collected from a farm in Kerman (Kerman Province, Iran) August 2002. The voucher specimens were identified by M. Mehrabani and deposited at the herbarium of the Kerman Faculty of Pharmacy, Kerman, Iran.

The plants were shade-dried and coarsely ground (mesh size: 20) before extraction. Plant material (50 g) was sequentially extracted by exhaustive maceration at room temperature with 1000 mL of methanol. The supernatants were strained through filter paper and evaporated to dryness under vacuum to obtain the methanol extract. A stock solution of the residues was made in methanol at a concentration of 10.24 mg/mL. The final amount of the crude plant extract was 4 mg/disk.

Disk diffusion method

The disk diffusion test was used for primary screening of the plant extracts against two clinical strains of *H. pylori*, a metronidazole-sensitive and a metronidazole-resistant strain from adult patients. Bacterial suspension, adjusted to yield approximately 1×10^9 cfu/mL, was streaked with a calibrated loop on plates of Muller–Hinton agar containing 7% defibrinated horse blood. Filter paper disks (6 mm diameter) were placed on the inoculated agar surfaces and impregnated with 40 μL of stock solutions. Pure methanol (40 μL) was used as a negative control. The plates were incubated for 5–7 days at 37°C under microaerophilic conditions.

All tests were performed in triplicate and the antibacterial activity was expressed as the mean of inhibition diameters (mm) produced by the plant extracts. Amoxicillin and metronidazole were used as positive control drugs.

Determination of minimum inhibitory concentration

The active extracts giving an inhibition zone ≥15 mm in diameter were chosen for further determination of the minimum inhibitory concentration (MIC) using the agar dilution method with Muller–Hinton agar containing 7% defibrinated horse blood according to the NCCLS (1998) guidelines (document M7-A4). Pure methanol was used in the assay as a negative control. A stock solution of each extract was serially diluted two-fold in methanol and 1 mL of each dilution was incorporated in 19 mL of melted agar medium and poured into a Petri dish. The final concentrations of the extracts in the medium ranged from 0.1024 to 0.032 mg/mL.

The agar plates were inoculated and then incubated for 5–7 days at 37°C under microaerophilic conditions. The MIC was defined as the lowest concentration of plant extract where there was no visible growth. All determinations were performed in triplicate and a growth control consisting of medium with methanol was included to ensure that the viability of the organisms was not affected by the solvent used to dissolve the plant extracts. For quality control and comparative analysis, the β-lactam antibiotic amoxicillin and metronidazole were also tested with each batch of plant extracts.

Results and discussion

The antimicrobial effects of many herbs have been well known for centuries. Many anti-*H. pylori* agents exhibiting a significant inhibitory effect have been identified from plant materials. The present study tested the anti-*H. pylori* activity of several plants from Iranian traditional medicine, which could be used as a useful source of novel drugs, thus supporting traditional regimens.

In the present work, extracts of 12 medical plants from Iran were assessed for their anti-*H. pylori* properties by using two different methods: the disk inhibition assay, based on diffusion of the plant extract in agar that measured growth inhibition, and an agar dilution assay that measured the MIC of the extracts. The results are presented in Tables 1 and Tables 2. Eight medicinal

Table 1. *In vitro* antibacterial activity of plant extracts against *Helicobacter pylori* using the disk diffusion method (final amount of crude plant extract=4 mg/disk).

Plant name	Plant part	Zone of inhibition (mm)	
		Metronidazole-sensitive strain	Metronidazole-resistant strain
Artemisia dracunculus	aerial parts	≤7	≤7
Bunium persicum	fruits	19	19
Carum carvi	fruits	10	12
Heracleum persicum	fruits	25	24
Myrtus communis	fruits	15	16
Nigella sativa	seeds	17	17
Pimpinella anisum	fruits	18	17
Salvia mirzayanii	leaves	30	28
Salvia officinalis	leaves	≤7	≤7
Teucrium polium	aerial parts	20	20
Trachyspermum copticum	fruits	9	10
Zataria multiflora	flowering tops	23	21
Amoxicillin (25 μg)		21	22
Metronidazole (4 μg)		16	≤7

Table 2. *In vitro* anti-*Helicobacter pylori* activity of plant extracts against 12 clinical isolates, expressed as minimum inhibitory concentration (μg/mL).

H. pylori strain	Plant								
	Bunium persicum	*Heracleum persicum*	*Myrtus communis*	*Nigella sativa*	*Pimpinella sativum*	*Salvia mirzayanii*	*Teurium polium*	*Zataria multiflora*	Metronidazole
H. pylori M22	128	128	256	256	128	64	128	128	8
H. pylori M12	128	128	512	128	128	64	128	128	16
H. pylori M31	256	128	128	128	256	64	256	256	16
H. pylori M40	256	128	128	512	256	64	256	64	8
H. pylori S22	128	64	64	256	128	32	256	256	2
H. pylori S35	256	32	256	128	512	32	128	256	4
H. pylori S42	256	64	512	256	128	64	256	128	2
H. pylori S45	128	128	512	256	256	64	128	256	4
H. pylori R19	512	128	256	256	64	64	512	64	>16
H. pylori R14	256	64	256	256	128	32	256	128	>16
H. pylori R42	128	64	256	256	256	64	256	128	>16
H. pylori R55	256	64	256	256	256	32	128	256	>16

plant extracts exhibited an inhibition zone of ≥15 mm, and were further evaluated for their effects on clinical isolates of *H. pylori* from adult patients.

Compared with amoxicillin and metronidazole, the extracts showed inhibitory effects on the growth of *H. pylori* at higher concentrations; however, the results suggest that the extracts have a considerable antibacterial activity against *H. pylori*. Among the plants tested, *S. mirzayanii*, *H. persicum*, *Z. multiflora*, and *T. polium* exhibited the strongest anti-*H. pylori* activities in the disk diffusion assay (zone of inhibition=20–30 mm).

Among the plant extracts evaluated for MIC, *S. mirzayanii* was the most active plant, with strong antibacterial activity against clinical isolates of *H. pylori* (MIC=32–64 μg/mL). *S. mirzayanii* is widely used in Iranian folk medicine. Decoctions of this plant have many purported medicinal properties and are used in folk medicine for the treatment of digestive disorders. A striking feature of the plant is the pleasant smell of the essential oil, present in numerous glands especially in the leaves.

Recently, it has been reported that the essential oil of *C. carvi* is weakly active against *H. pylori* (Bergonzelli et al., 2003), which corroborates our results. Another previous investigation of a methanol extract of the seeds of this plant reported the MIC value as 100 μg/mL, also indicating weak activity (Mahady et al., 2005).

Previous investigators have reported that an extract obtained from the aerial parts of *T. copticum* inhibited the growth of *H. pylori in vitro* (Nariman et al., 2004). However, our results showed that the extract of the fruits of this plant had only very weak anti-*H. pylori* activity.

While some investigators have reported the susceptibility of *H. pylori* to extracts from the leaves of *S. officinalis* (Mahady et al., 2005; Nostro et al., 2005), there was almost no anti-*H. pylori* activity in the plant extracts of the present study. These data may differ owing to the region where the leaves were collected,

the extracts themselves, and the potential difference in plant chemistry of different samples.

In conclusion, the present results showed a significant *in vitro* susceptibility of clinical strains of *H. pylori* to extracts of medicine plants used in Iranian traditional medicine. Thus, these plants may be considered a potential new source of agents for the treatment of *H. pylori* infections, and may be further developed for new and safe agents for inclusion in anti-*H. pylori* regimens. Further studies are needed to investigate the effects of these active plant extracts and determine the chemical constituents in the extracts responsible to allow for *in vivo* studies.

***Declaration of interest*:** The authors report no conflicts of interest.

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